In re Application of:

Epstein et al.

Application No.: 10/618,183

Filed: July 10, 2003

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Attorney Docket No.: MEDIV2010-4

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Upon entry of the present amendment, the claims will stand as follows:

Please cancel claims 1, 2, 4-8, 13, 14-16, 19, 20-23, 33, 35-38,

Please amend claims 17, 19, 24, 25, 29-32, 34, 39, 40, 43 and 46 as follows:

17. (Currently Amended) A method for enhancing collateral blood vessel formation in heart or limb muscle tissue of a patient in need thereof, said method comprising:

obtaining autologous bone marrow from the patient;

growing the autologous bone marrow in a suitable medium under suitable culture conditions for a period of time sufficient to promote production by the bone marrow of early attaching cells;

transfecting at least a portion of the early attaching cells with an adenovirus vector comprising a polynucleotide that encodes one or more agents selected from hypoxia inducing factor 1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte monocyte colony stimulatory factor (GM-CSF), a fibroblast growth factor (FGF), a NOS, and PR39 so as to cause expression of the one or more agents to produce conditioned medium; and

directly administering to injecting into a site of impaired blood flow in heart or limb muscle tissue of the patient an effective amount of the transfected early attaching cells and/or the conditioned medium to enhance collateral blood vessel formation at the site in the patient obtained from autologous bone marrow, which cells contain an adenoviral vector comprising a polynucleotide encoding one or more angiogenic factors selected from hypoxia inducing factor-1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte-monocyte colony stimulatory factor (GM-CSF), PR39, a fibroblast growth factor (FGF), and a nitric oxide synthase (NOS).

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Claim 18 (Cancelled)

19. (Currently Amended) The method of claim [[18]] <u>17</u>, wherein the early attaching cells are marrow-derived stromal cells and the <u>conditioned medium composition</u> is directly administered to a site of ischemia in the <u>patient muscle tissue</u>.

Claims 20-23 (Cancelled)

- 24. (Currently Amended) The method of claim [[23]47, wherein the period of culturing is from about 3 hours to about 3 days.
- 25. (Currently Amended) The method of claim [[18]] <u>17</u>, further comprising filtering the bone marrow prior to culturing of the bone marrow to obtain the early attaching cells.

Claims 26 - 28 (Cancelled)

- 29. (Currently Amended) The method of claim [[18]] <u>17</u>, wherein the agent is selected from a fibroblast growth factor (FGF), a NOS, and PR39.
- 30. (Currently Amended) The method of claim [[29]] 17, wherein the agent is selected from FGF-1, FGF-2, FGF-4, and FGF-5.
- 31. (Currently Amended) The method of claim [[29]] 17, wherein the agent is selected from inducible NOS and endothelial NOS.
- 32. (Currently Amended) The method of claim [[29]] 17, wherein the agent is PR39.
- 33. (Cancelled)

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34. (Currently Amended) The method of claim [[18]] 17, wherein the method enhances collateral blood vessel formation in the heart or leg muscle tissue is heart or limb muscle tissue.

Claims 35 – 38 (Cancelled)

- 39. (Currently Amended) A therapeutic composition comprising early attaching cells derived obtained from bone marrow, which cells have been transfected with contain an adenoviral vector comprising a polynucleotide that encodes one or more agents factors selected from hypoxia inducing factor-1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte-monocyte colony stimulatory factor (GM-CSF), PR39, a fibroblast growth factor (FGF), and a nitric oxide synthase (NOS).
- 40. (Currently Amended) The therapeutic composition of claim 39, further comprising conditioned medium in which the cells have been grown in culture for a time sufficient to allow expression of containing one or more of the agents factors expressed therein.
- 41. (Original) The composition of claim 39, wherein the polynucleotide further comprises a transcription regulatory region operatively associated with the polynucleotide.
- 42. (Original) The composition of claim 39, wherein the transfected cells have been stimulated by exposure to hypoxia.
- 43. (Currently Amended) The composition of claim 39, further comprising heparin or another an anticoagulant.
- 44. (Cancelled)

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45. (Original) The composition of claim 39, wherein the early attaching cells are marrow-derived stromal cells.

46. (Currently Amended) The composition of claim 39, wherein the composition is intended to be injected into a patient donor having ischemic tissue and the early attaching cells are derived from bone marrow obtained from the patient donor.

Please add the following new claims:

- 47. (New) The method of claim 17, further comprising, prior to the injecting, culturing the early attaching cells in a culture medium to produce the one or more agents in conditioned medium, and wherein the composition further comprises the one or more agents in the conditioned medium.
- 48. (New) The method of claim 17, wherein the injecting is at multiple sites in the muscle tissue.
- 49. (New) The method of claim 48, wherein the effective amount is about 0.2 to about 0.5 ml of the composition in each of from about 12 to about 25 sites.